

Challenges in Characterizing and Identifying Components in Botanical Products

Joseph M. Betz

Office of Dietary Supplements

U.S. National Institutes of Health

Department of Health and Human Services

<http://dietary-supplements.info.nih.gov/>

March 25, 2005



Disclaimer

SHOE JEFF MACNELLY



- Views expressed are my own and do not reflect the views of ODS, NIH, or HHS



Known Bioactive Compounds in Foods

➤ Inherent to Foods

- Alkaloids
- Cyanogens
- Glucosinolates
- Lectins
- Carbohydrase Inhibitors
- Vicine/Convicine (Favism)
- Phenolics
- Non-protein Amino Acids
- Peptides

➤ Accidental Ingestion

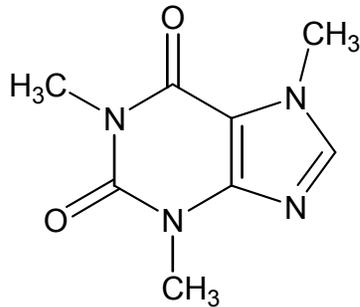
- Mushroom Toxins

➤ Contaminants in Foods

- Mycotoxins
- Alkaloids
- Shellfish toxins



Alkaloids



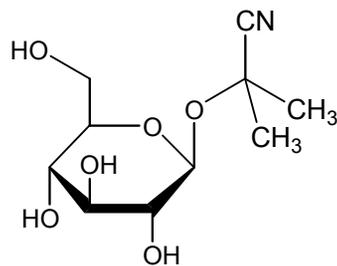
Caffeine-

**LD50-127 mg/kg* mice,
50-500 mg/kg*, humans**





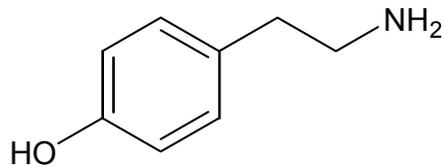
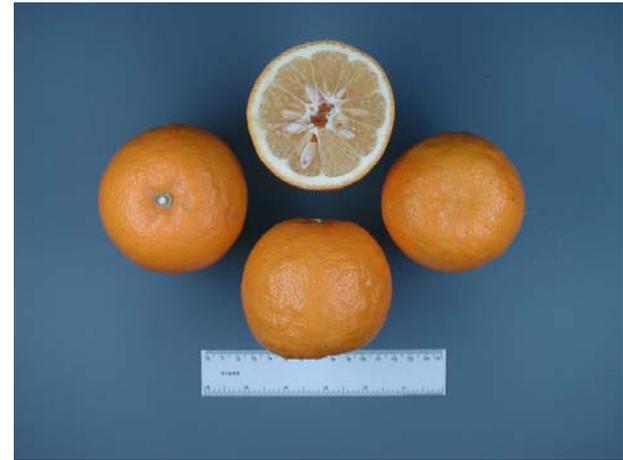
Cassava (manioc)- *Manihot esculenta*



linamarin

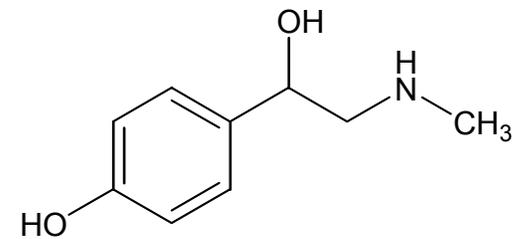
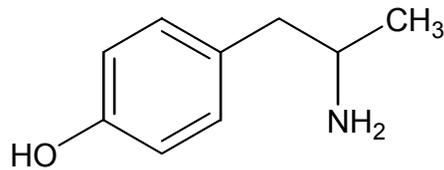


Biogenic Amines

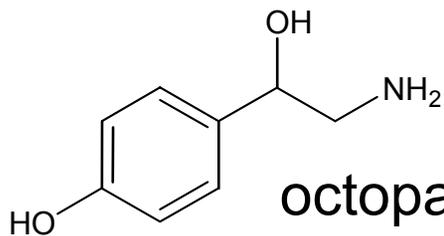


tyramine

N-methyltyramine



synephrine



octopamine

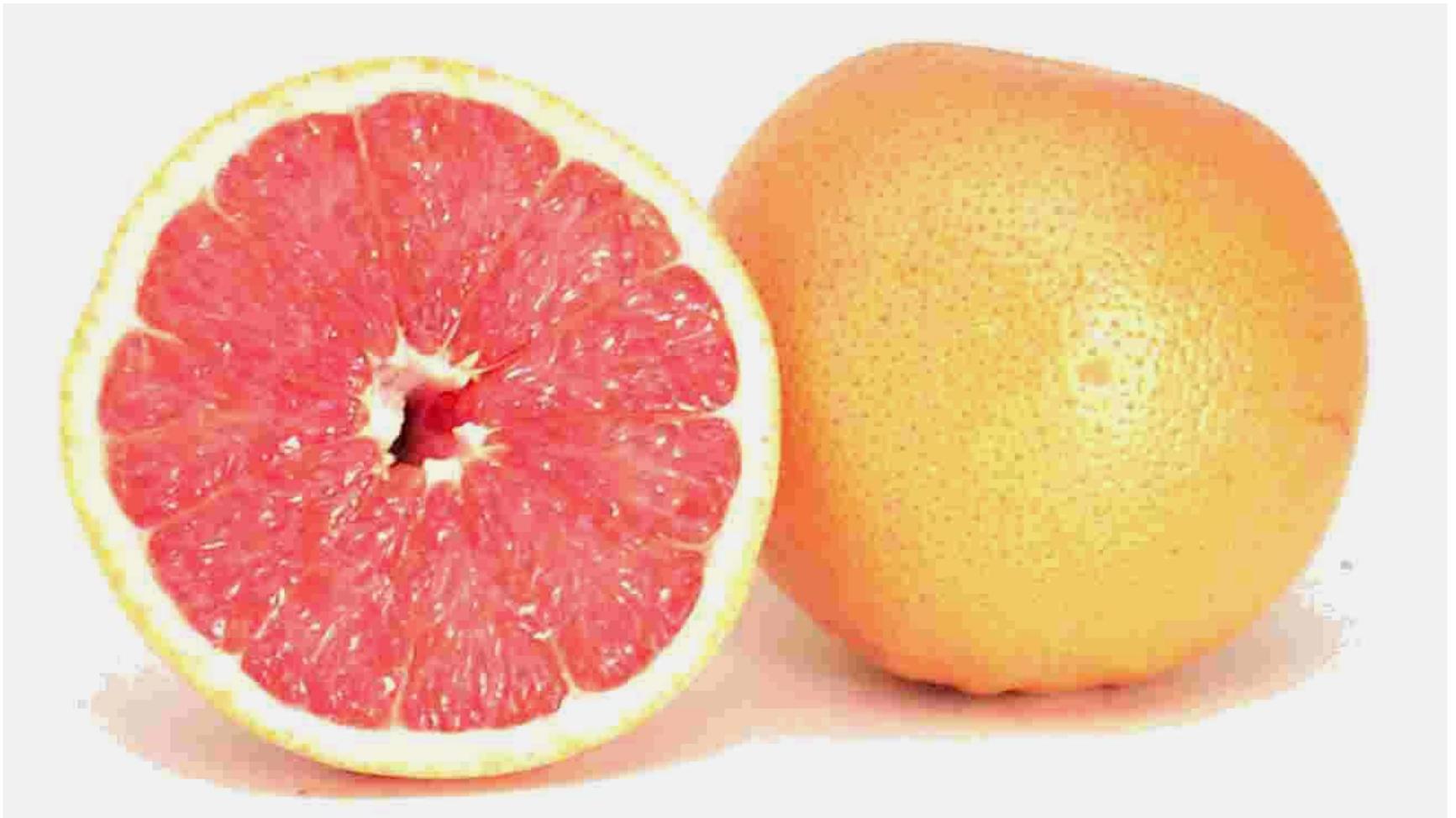


Biogenic Amines

	synephrine	tyramine	Me-tyramine	Octapamine
<i>C. reticulata</i> (tangerine)	125	1	15	1
<i>C. reticulata</i> (Mandarin or.)	280	1	58	2
<i>C. aurantium</i> (sour orange)	19	-	1	-
<i>Musa paradisiaca</i> (banana)	-	29	-	-

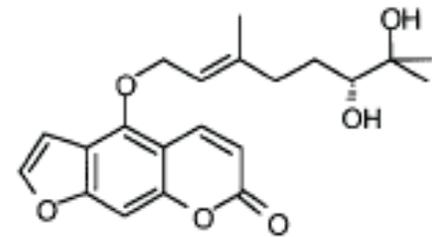
Mg/kg fresh



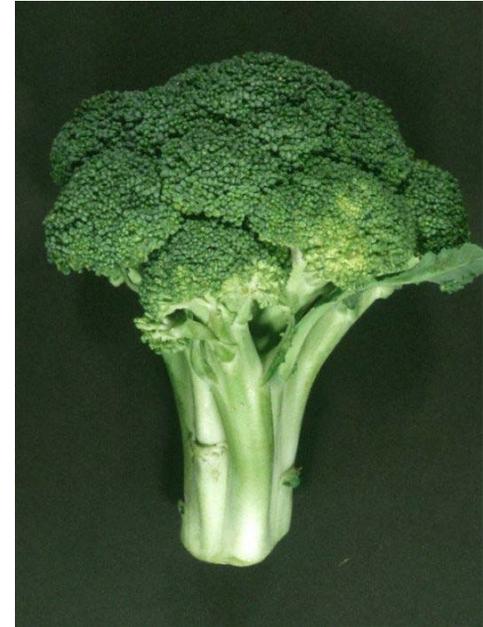
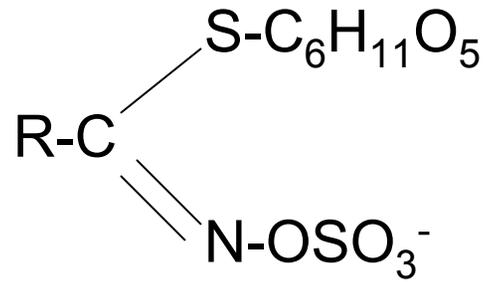


6'7'-Dihydroxybergamottin

Grapefruit juice inhibits intestinal cytochrome
P450 (CYP) 3A4



Glucosinolates



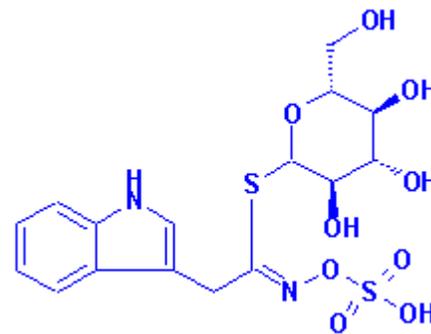
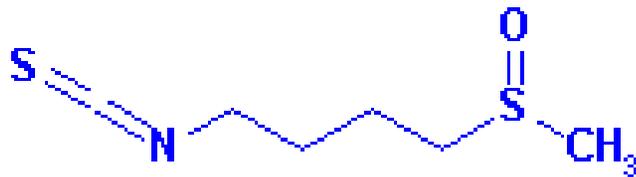
Glucosinolates

3-Methylsulfinylpropyl-glucosinolate: Glucoiberin

Allyl-glucosinolate: Sinigrin

4-Methylsulfinylbutyl-glucosinolate: Glucoraphanin

3-Indolylmethyl-glucosinolate: Glucobrassicin



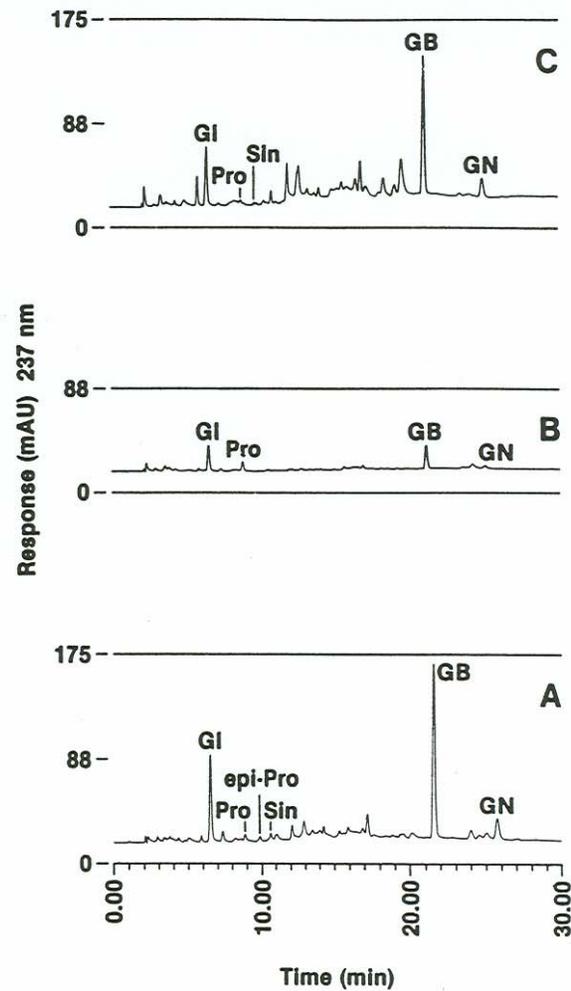
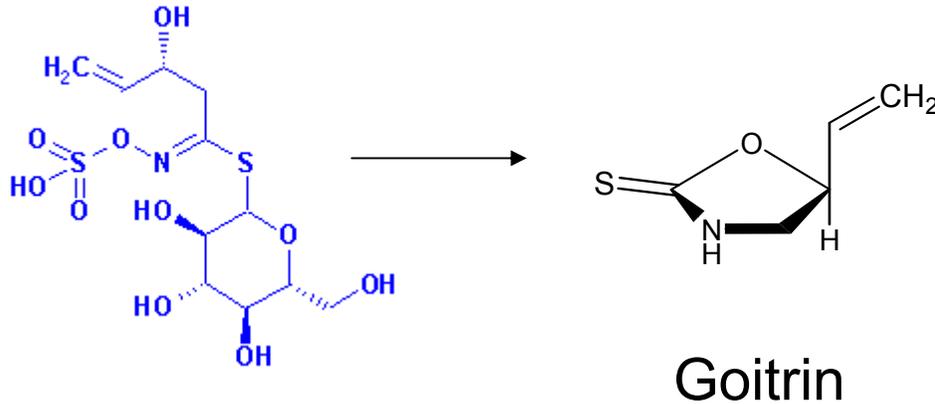


Figure 2. Representative chromatograms of A) fresh raw, B) boiled 5 min, and C) stir fried broccoli. GI = glucoiberin; Pro = progoitrin; *epi-Pro* = *epi-progoitrin*; Sin = sinigrin; GB = glucobrassicin; GN = gluconasturtiin.



Glucosinolates



Progoitrin

Hepatic, renal,
Pancreatic lesions
caused by OZT's and
nitriles

Goitrin

Thiocyanates and L-5-vinyl-2-thioxazolidone=Goitrogens

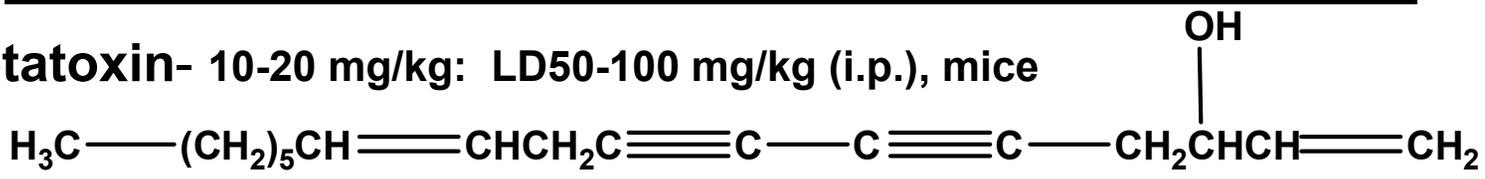
- Activity of goitrin not reversible by dietary iodine





© W.P. Armstrong 2001

Carotatoxin- 10-20 mg/kg: LD50-100 mg/kg (i.p.), mice



Fabaceae

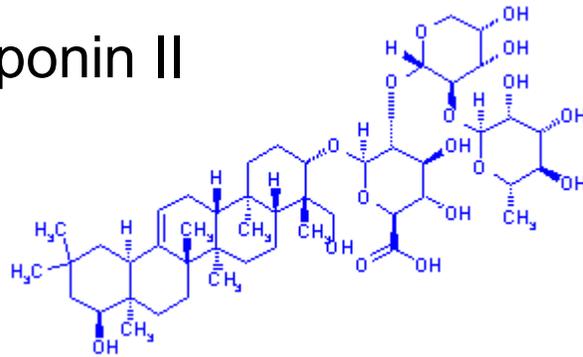


Glycine max

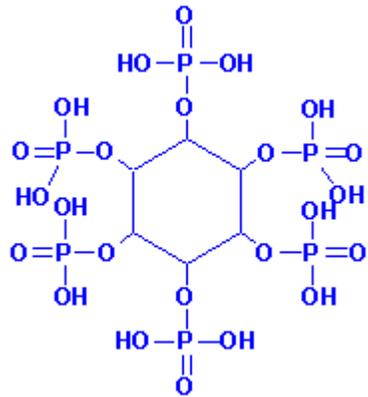
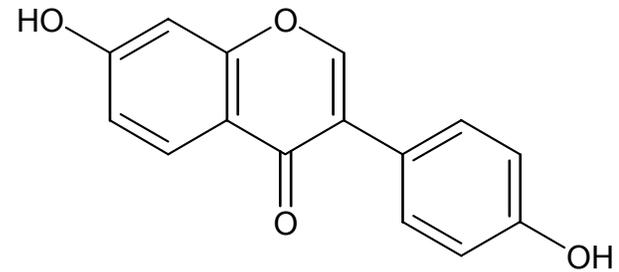


Soy (Glycine max)

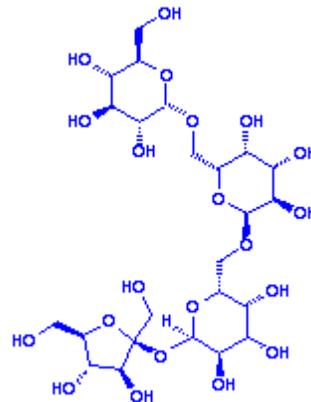
Soyasaponin II



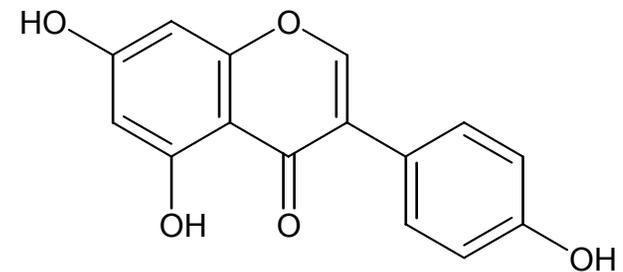
Daidzin=daidzein-7-glucoside



Stachyose



Genistin=genistein-7-glucoside



Phytic Acid

Bowman-Birk
Protease Inhibitor

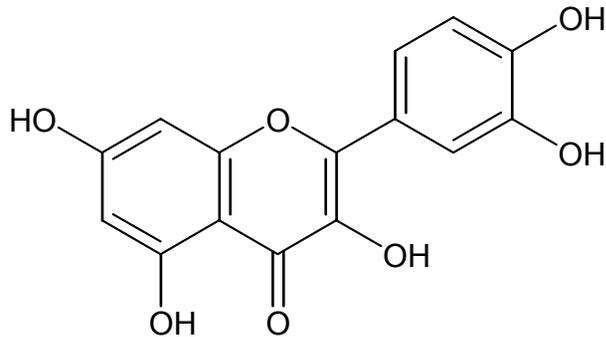


**"What we observe
is not nature itself,
but nature exposed
to our method of
questioning."**

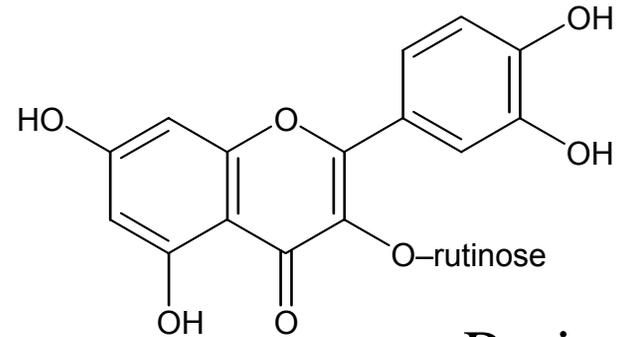
*- Werner
Heisenberg*



Biological relevance



Quercetin



Rutin

Compound	Rel. Efficiency
THBQ	1.00
BHA	1.67
Quercetin	0.18
Rutin	0.01

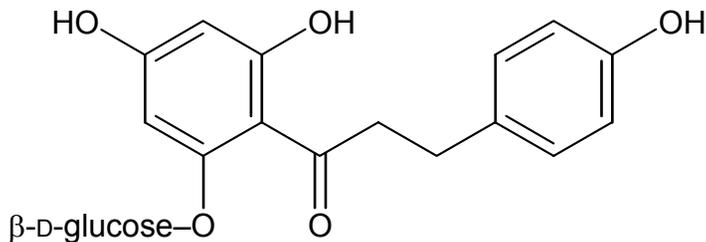


Cytotoxicity in Caco 2 cells

(Inhibition of MTT reduction)

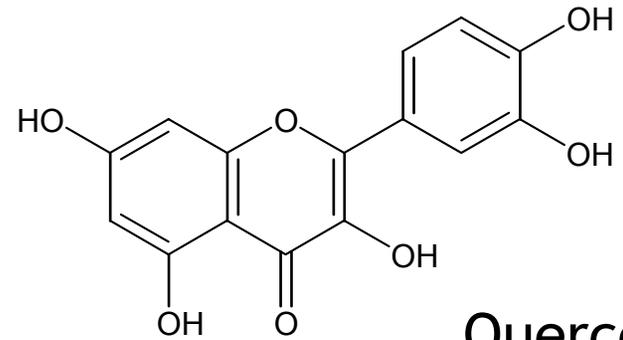
AJE	Quercetin	Rutin	Phloridzin
1 mM	50 μ M	5 μ M	5 μ M

- Addition of ascorbate or α -tocopherol abolished cytotoxicity
- Postulated that formation of reactive polyphenol oxidation products at high levels responsible for cytotoxicity
- Also postulated that AJE was less cytotoxic because the compounds protected each other



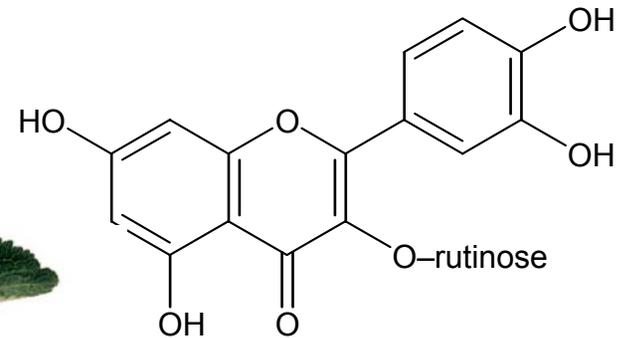
Phloridzin

Pohl et al. 2004 *The Toxicologist* 78,S-1: 213

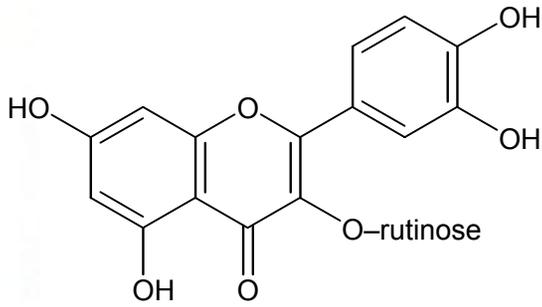


Quercetin

Rutin

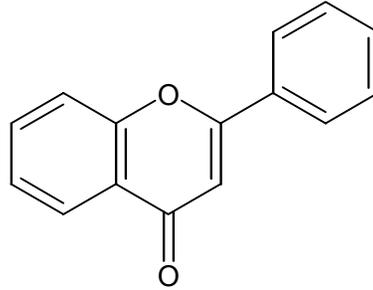


Ubiquitous plant flavonoids

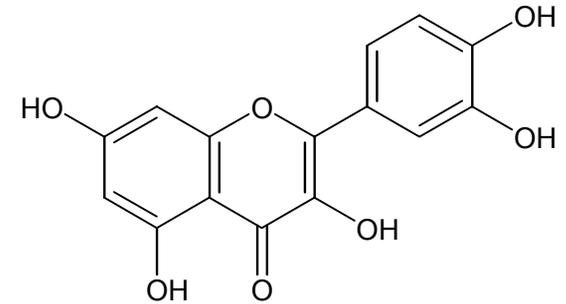


Rutin

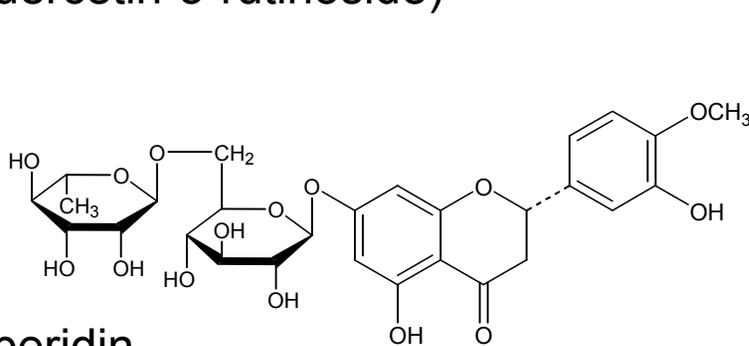
(quercetin-3-rutinoside)



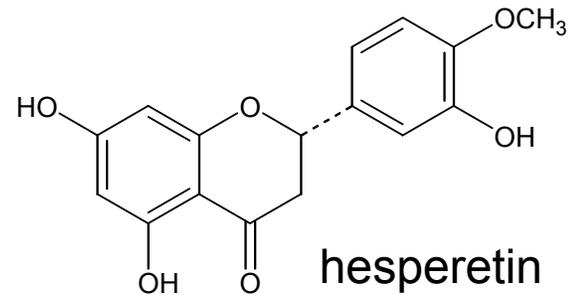
Flavone



quercetin



hesperidin



hesperetin

Hydroxylated flavonoids (quercetin, naringenin) usually inhibit P450 isozymes *in vitro*, nonhydroxylated flavonoids (flavone, nobiletin, tangeretin) stimulate P450 system *in vivo* and *in vitro*



Quercetin

- Quercetin reduces expression of the amiloride-sensitive epithelial Na⁺ channel (ENaC) in salt sensitive hypertensive rats (Aoi et al. 2004 *Biochem Biophys Res Commun* **315**:892)
 - ENaC is rate limiting step in Na⁺ reabsorption in distal segment of renal tubule
 - High-salt diet rats have elevated α ENaC expression and BP
 - Quercetin blocked elevated expression of α ENaC mRNA and BP elevation
 - Quercetin effect on α ENaC not found in colon
 - Aglycone (colon) vs. glucuronide, sulfate after absorption?



Quercetin

- Quercetin (10 μ M) inhibits insulin-stimulated methylglucose uptake by direct interaction with the facilitative glucose transporter GLUT4 in rat adipocytes (Strobel et al. 2005 *Biochem. J.* **386**:471)
 - Does not inhibit protein tyrosine phosphorylation
- Quercetin non-competitively antagonizes the γ -aminobutyric acid C receptor subunit GABA ρ_1 (Goutman & Calvo, 2004 *Brit J Pharmacol* **141**:717)
 - Quercetin active at ionotropic GABA receptors and other ligand-gated ion channels





St. John's Wort

- 1814-1st American mention of toxicity of *H. perforatum* (to livestock)
- 1° photosensitivity
 - hypericin + light = lipid hydroperoxide *in vitro*
- SJW induced neuropathy, erythroderma
 - Hypericin-induced phototoxicity *in vitro*
 - long term effects?
- SJW/Indinavir
 - Herb/drug interactions



Hypericum perforatum L.

- DAC '86 (German Drug Codex)- not < 0.4% dianthrones of hypericin group, as hypericin
- DAC '91: method change to correct for chlorophyll interference: results are 20% lower
- Hypericin MAO inhibitor *in vitro*
- 1996 trial showed hypericin-free but not hyperforin free products effective
- USP '99-SJW extract, not < 0.2% hypericin and pseudohypericin combined , not < 3.0% hyperforin (LC)

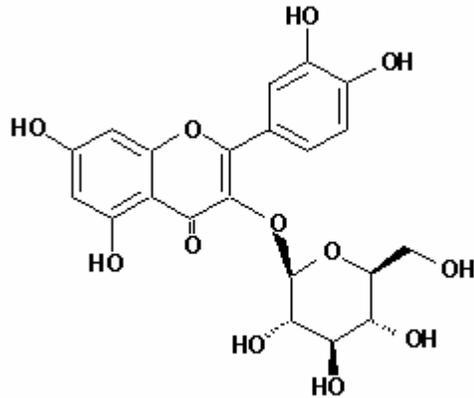


Are we having fun yet?

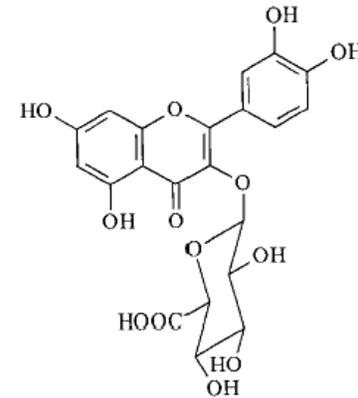
- *Bioassay directed H. perforatum* prep HPLC
- Flavonoid rich fractions-Antidepressant (FST)
 - ⇒ Hyperoside, isoquercitrin, miquelianin, quercitrin, some hyperoside, astilbin
 - ⇒ Isolated pure compounds tested
- All pure cmpds except quercetin, quercitrin, and astilbin active in FST (Butterweck *et al.* (2000) *Planta Med.* **66**:3-6)
- 2002: new proprietary SJW extract product



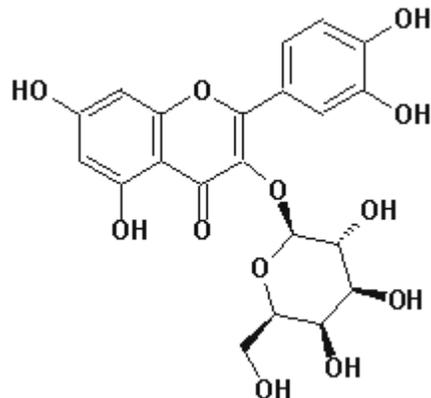
SJW flavonoids



isoquercitrin
(quercetin 3-O- β -D-glucopyranoside)



miquelianin
(quercetin 3-O- β -D-glucuronopyranoside)



hyperoside
(quercetin 3-O- β -D-galactopyranoside)



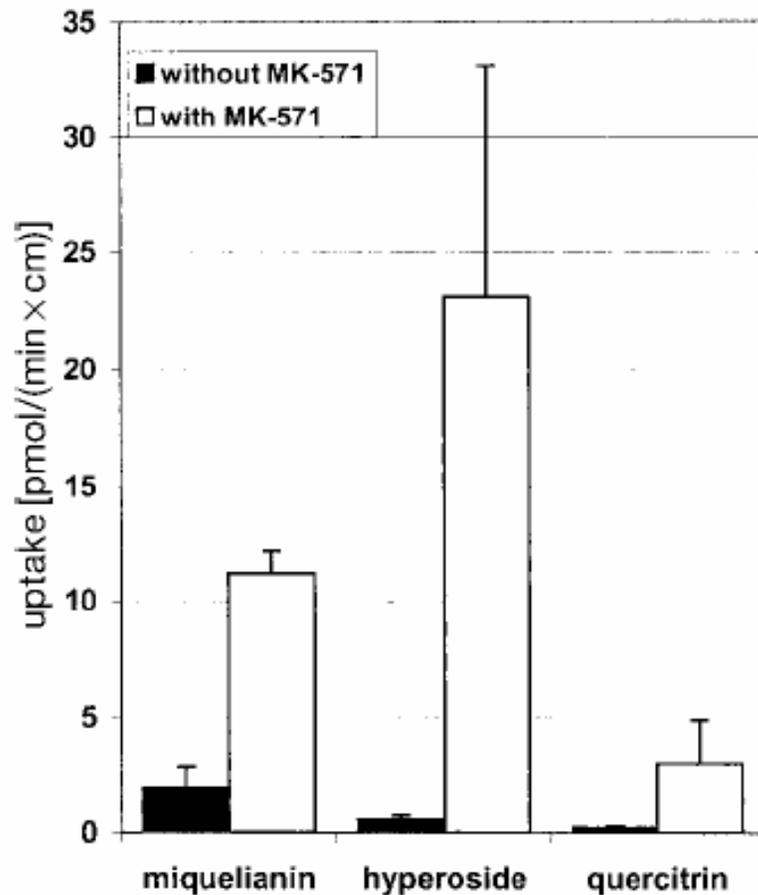


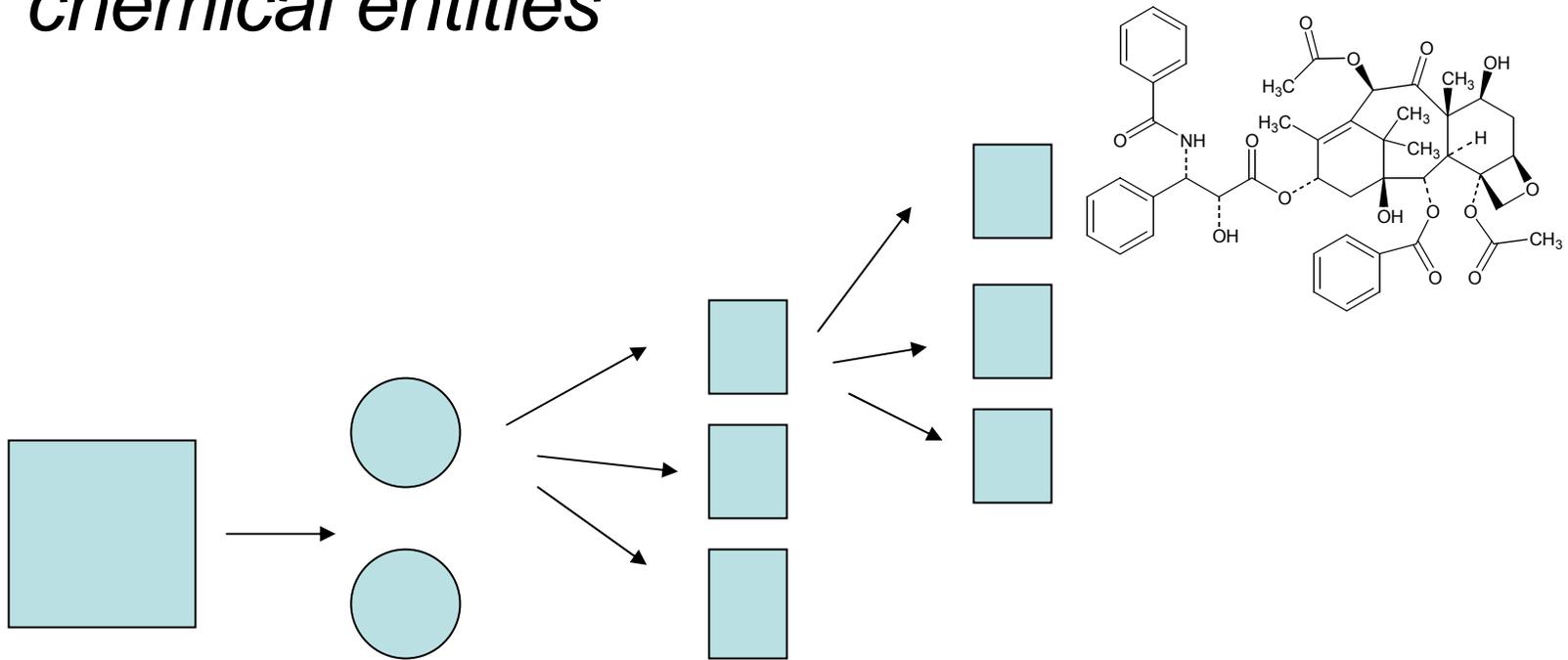
Fig. 1 Uptake of different flavonoid-glycosides into Caco-2 cells with and without 50 μ M MRP-2 inhibitor MK-571 (mean \pm SE; n = 3 - 4).

Juergenliemk G et al. In vitro-studies indicate... *Planta Med* 2003; 69: 1013 - 1017



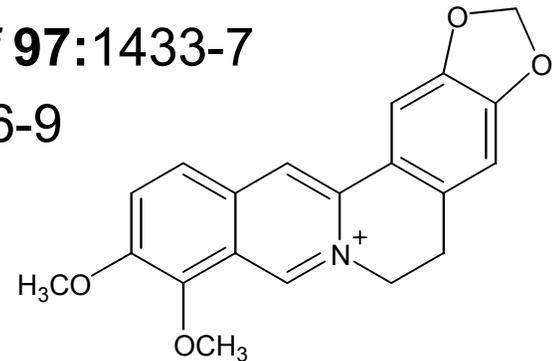
Drug Discovery

- *Natural Products Cancer Drug Discovery*
- *Bioassay Directed Fractionation to pure chemical entities*



“Whole plant”

- *Berberis* spp.-marker=berberine
- Flavonolignan: 5'-methoxyhydnocarpin (5'-MHC) inhibitor of the MDR pump in *S. aureus*
- Prevents bacterial cells from eliminating berberine
 - Stermitz *et al.* (2000) *Proc Natl Acad Sci* **97**:1433-7
 - Stermitz *et al.* (2000) *J Nat Prod* **63**:1146-9





"OF COURSE YOU CAN'T REPLICATE MY
EXPERIMENTS. THAT'S THE BEAUTY OF THEM."

Analytical Methods and Reference Materials Program

■ Quercetin dihydrate (7)

Molecular Formula: $C_{15}H_{10}O_7 \cdot 2H_2O$, Formula Weight: 338.27,
CAS Number: 6151-25-3

- [Q0125](#) $\geq 98\%$ (HPLC), powder (Sigma)
- [Q-112](#) solid (Sigma)
- [83370](#) *BioChemika*, $\geq 98.0\%$ (HPLC) (Fluka)
- [33,795-1](#) $\geq 95\%$ (Aldrich)
- [17,196-4](#) 98% (Aldrich)
- [32782](#) $> 99\%$ (Riedel-de Haën)
- [69249](#) $\geq 99.0\%$ (HPLC) (Fluka)



Quercetin Dihydrate

(1200 mg)

Company	HPLC Purity (%)	Water Content (%)	Residual Content (%)	Calculated Value (%)	Price
A	99.57	20.2	0	79.46	\$550
B	99.46	0.41	0.4	99.12	\$6500
C	ca 90				\$1250
D	Not less than 98	NA	NA		\$1320

